

A bovine hemoglobin-based oxygen carrier as pump prime for cardiopulmonary bypass: reduced systemic lactic acidosis and improved cerebral oxygen metabolism during low-flow in a porcine model

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## **Abstract**

**Objectives:** Cerebral ischemia can occur during cardiopulmonary bypass, especially during low-flow. HBOC-201 is a hemoglobin-based oxygen carrying solution that enhances oxygen delivery. This project evaluated benefits on total body and cerebral oxygen delivery and consumption using HBOC-201 during cardiopulmonary bypass.

**Methods:** 12 immature swine were assigned to one of two groups. One group utilized HBOC-201 in pump prime, the other utilized donor porcine blood. Cardiopulmonary bypass was initiated; then flow was serially decreased from 100% to 75%, 50% and then back to full flow. At each interval,  $^{15}\text{O}$  positron emission tomography was performed and blood collected. Total body and cerebral oxygen delivery and consumption were calculated. Statistical analysis was performed using a Tukey-Kramer adjusted p-value based on repeated measure linear model on log transformed data.

**Results:** Total and plasma hemoglobin were higher in the HBOC-201 group. Oxygen delivery and consumption were not statistically different, but did tend to be higher in the HBOC-201 group. Mixed venous saturation was lower in the HBOC-201 group, but not significant. Mild metabolic acidosis with elevated lactate developed in the Blood group. Mean cerebral blood flow decreased in both groups when total flow was 50%. In the HBOC-201 group, the cerebral oxygen metabolism was maintained.

**Conclusions:** The addition of HBOC-201 for cardiopulmonary bypass appears to improve oxygen utilization and minimize anaerobic metabolism. Cerebral oxygen utilization was preserved in the HBOC-201 group even during decrease in blood flow. These findings support reported improved oxygen unloading properties of HBOC-201 and may provide a benefit during cardiopulmonary bypass.

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## Mini-abstract

In a porcine model, HBOC-201 was added to the priming solution during cardiopulmonary bypass. HBOC-201 reduced overall anaerobic metabolism and maintained cerebral oxygen metabolism compared to donor blood during normothermic low-flow cardiopulmonary bypass.

## **Background**

HBOC-201 (OPK Biotech, Cambridge, MA), is a bovine hemoglobin-based oxygen carrier (HBOC). Developed as red cell replacement, these fluids are being evaluated as oxygen therapeutics. HBOCs have characteristics that are beneficial during tissue ischemia. Hemoglobin in solution increases the oxygen carrying capacity of plasma phase, decreases the oxygen diffusion resistance, and facilitates oxygen delivery to tissue beds (1,2). HBOC-201 is a “high  $P_{50}$ ” HBOC: the  $P_{50}$  of HBOC-201 is 38 mm Hg compared to 27 mm Hg for human intra-erythrocytic hemoglobin (see Table 1). This decreased affinity enhances oxygen offloading to tissues (3-5).

Prior work (6-9), utilizing a porcine model of controlled hemorrhagic shock demonstrated the potential benefits of the enhanced tissue oxygenation. In acute and survival models, resuscitation with HBOC-201 led to anaerobic metabolism reversal despite dramatically lower cardiac output, mean arterial pressure, and mixed venous saturation without significant end-organ injury. In these studies, despite lower oxygen delivery and systemic hypotension, oxygen utilization was maintained and end-organ function preserved.

$^{15}\text{O}$  Positron Emission Tomography (PET) measures cerebral blood flow (CBF) and cerebral oxygen metabolism ( $\text{CMRO}_2$ ) and has become the gold standard (15, 16) for cerebral perfusion studies. No studies using  $^{15}\text{O}$  PET imaging during cardiopulmonary bypass (CPB) have been published, but  $^{15}\text{O}$  PET has been used to evaluate cerebral metabolism in a porcine model after cardiac arrest (17). For brain imaging, major advantages of PET using  $^{15}\text{O}$  (half-life = 122.2 seconds) are that the images are rapidly acquired, fully quantitative, and can be repeated every ten minutes. A limitation of using  $^{15}\text{O}$  is that an on-site cyclotron is required

to generate the required oxygen radiotracers. Few medical research facilities have the required equipment, precluding widespread application.

Neurological complications can occur after surgery requiring CPB. The common pathway is cerebral ischemia leading to neuronal death. Because of the facilitated oxygen delivery and reduced tissue ischemia during low flow, we hypothesized that HBOC-201 during CPB may improve cerebral protection by minimizing overall end organ ischemia. No prior investigations have evaluated this hypothesis. However, other clinical situations with end-organ ischemia have been evaluated. In myocardial infarction models, prophylactic and therapeutic treatment with HBOC-201 led to significant infarct size reduction (10,11). Porcine models of hemorrhagic shock combined with traumatic brain injury (12,13) using HBOC-201 for resuscitation demonstrated rapidly restored hemodynamics and improved brain tissue oxygenation. A canine model of isovolemic hemodilution found tissue oxygenation in both skeletal muscle and liver parenchyma was raised above baseline values (14) in the HBOC-201 group. Therefore, we measured CBF and CMRO<sub>2</sub> in a porcine model of normothermic low-flow CPB.

## **Methods-**

### Animal Model

This study was reviewed and approved by the Wilford Hall Medical Center Animal Care and Use Committee. All animals were cared for according to the *Guide for the Care and Use of Laboratory Animals* (18). Twelve Yorkshire swine (wt 23.3 ± 3.3 kg) were randomized to 2 groups. Animals were fasted but had access to water. Animals were premedicated with intramuscular ketamine (15-20 mg/kg) and atropine (0.04-0.4 mg/kg).

Intravenous access was established. Mask induction using isoflurane (2.0 – 4.0%) in 100% oxygen was performed followed by endotracheal intubation. Anesthesia was maintained with isoflurane (0.25-2.0%) in 100% oxygen and the animals were mechanically ventilated with PEEP = 3 cm H<sub>2</sub>O. Femoral intravascular catheters were placed via cutdown. Median sternotomy and systemic heparinization (300-500U/kg, IV) was performed. Activated coagulation time (ACT) was maintained >450 sec. A venous cannula was placed through the right atrial appendage. An arterial cannula was placed in the ascending aorta. The left atrium was vented through the appendage. The aortic arch was mobilized and the ligamentum arteriosum ligated. A fiber-optic positron ( $\beta^+$ ) probe was placed in the right brachiocephalic artery and advanced into the right common carotid artery.

The animals were transferred from the surgical suite to the PET scanner and the cannulae were connected to the bypass circuit. Positioning for PET scanning was accomplished, baseline measurements were recorded and blood samples collected. Prior to initiating CPB, additional anesthetic with fentanyl (30-100  $\mu$ g/kg/hr) and neuromuscular blockade with pancuronium bromide (0.02-0.15 mg/kg, IV) was administered. Isoflurane (0.25-2.0%) was continued via a vaporizer on the CPB circuit.

### Experimental Protocol

In group A, HBOC-201 was added to the standard priming solution (12.5 gm mannitol, 50 mEq NaHCO<sub>3</sub>, 10000 units heparin in isotonic saline) to produce a systemic serum concentration of free hemoglobin of 2gm/dL using the formula:

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$$\text{EBV} = \text{estimated blood volume (70mL/kg), circuit volume} = 800 \text{ mL, [Hg] in HBOC} = 13 \text{ g/dL}$$

In group B, the same prime with a similar volume of donor porcine whole blood was utilized. Other than this difference in the priming solution, both groups were treated identically. The bypass circuit consisted of an S-3 roller head bypass system (Sorin Medical, Denver, CO) with a Capiiox RX15 oxygenator, AF125X arterial filter, and HC05 hemoconcentrator (Terumo Medical, Somerset, NJ), using 3/8" tubing for arterial and venous lines. Full flow normothermic CPB (100mL/kg/min) was initiated and maintained for 30 minutes, the flow was decreased to 75% for 30 minutes, 50% for 30 minutes, and then returned to full flow for 30 minutes. Animals were separated from CPB, observed for 30 minutes, then euthanized. Cardioplegic arrest was not performed. Vacuum assisted venous drainage and venting of the left atrium were performed to optimize venous drainage and assure that no ventricular ejection contributed to cardiac output. Hemodynamic support with dopamine infusion was utilized as necessary. Heparinization was not reversed and cannulae were left in place throughout all data acquisition. At each interval,  $^{15}\text{O}$  PET imaging was performed and arterial and venous blood samples were collected. Arterial blood pressure was monitored continuously via the femoral arterial line and heart rate was monitored by continuous EKG. Core temperature was measured by rectal temperature probe. CPB flow was measured via ultrasonic flow probe on the arterial inflow tubing and recorded at each interval.

$^{15}\text{O}$  tomography was performed on a research-dedicated Siemens/CTI HR+ scanner (Siemens/CTI, Knoxville, TN, USA) in 2-D acquisition mode to reduce scattered-photon counts. For calibration, a blank scan was performed before the animals were placed on the scanner. For each animal, a 10 minute transmission scan was done with a rotating rod source of  $^{68}\text{Ge}$  to correct the subsequent emission scans. For CBF measurement, 1480 MBq of



H<sub>2</sub><sup>15</sup>O in 6 ml of saline was injected as an intravenous bolus beginning 20 seconds before scan initiation. Cerebral oxygen metabolic rate (CMRO<sub>2</sub>) was measured using 4810 MBq of <sup>15</sup>O<sub>2</sub> in a 2L air bag delivered across the CPB oxygenator membrane. Arterial input function (AIF) was measured using a β<sup>+</sup> probe system developed by Lee, *et al.* (19) The intra-arterial β<sup>+</sup> probe was placed into the common carotid artery and a second probe was placed outside the body to remove background gamma radiation. PET images were reconstructed using a standard filtered back-projection algorithm with a 5 mm FWHM Hann filter. Decay and dead time corrections were done during image reconstruction. The images were co-registered by anatomical MRI images. Absolute whole-brain CBF (ml/100g/min) and CMRO<sub>2</sub> (ml/100g/min) were calculated using Ohta method for different bypass stages. Cerebral oxygen extraction fraction (OEF) was estimated from the relationship between CBF and CMRO<sub>2</sub> (i.e., CMRO<sub>2</sub> = CBF x OEF x oxygen content). (20)

At each interval, arterial and mixed venous blood was collected and analyzed using an on-site Radiometer ABL 510 (Copenhagen, Denmark) for standard blood gas values, hemoglobin, co-oximetry, and lactate levels. Using this data, the arterial and venous oxygen content was calculated. Oxygen delivery, oxygen consumption, and oxygen extraction ratio was calculated at each bypass flow rate. At baseline and after separation from CPB, blood was collected and stored for subsequent analysis. Complete cell count was performed on an ADVIA analyzer (Seimens Healthcare, Deer Park, IL) after completion of each experiment. Standard serum chemistries were performed using a Vetscan analyzer (Abaxis, Union City, CA). Baseline and completion serum samples were analyzed on a Radiometer ABL 510 (Copenhagen, Denmark) for the concentration of cell-free hemoglobin. Specimens for inflammatory mediators (TNF-α, IL-6) were collected, stored at -78° C, and subsequently

analyzed in batch utilizing ELISA kits (R&D Systems, Minneapolis, MN). Similarly, troponin (Life Diagnostics, Westchester, PA) and CKMB (Diagnostic Automation, Calabasas, CA) levels were determined using ELISA kits on stored specimens in batch.

Data were summarized with the mean  $\pm$  one standard deviation in original units. Mean contrasts were carried out using a Tukey-Kramer adjusted p-value based on a repeated measures linear model on log transformed data with a compound symmetric covariance assumption. All statistical testing was 2 sided with an experiment-wise significance level of 5%. SAS Version 9.2 for Windows (SAS Institute, Cary, North Carolina) was used throughout.

## **Results-**

All animals survived to completion of the protocol with the exception of one animal which was excluded due to loss of airway with hemodynamic compromise. During transfer, this animal became extubated leading to significant hypoxia and hypercarbia with subsequent hemodynamic compromise. The data from this animal were discarded and the animal was not replaced. One animal developed acute hind-limb ischemia secondary to arterial thrombosis at the femoral arterial catheter site. Hemodynamic and PET data were used from this animal, but metabolic and inflammatory markers that would have been influenced by the extremity ischemia were not included in the data analysis. The mean weights of the animals in both groups were not significantly different. The mean volume of HBOC-201 used for each animal was  $372 \pm 24$  mL or  $16.2 \pm 0.4$  mL/kg.

### Hemodynamics

In the HBOC group, the mean arterial pressure(MAP) was higher and the mean heart rate remained lower (Figure 1). No animals required the use of dopamine during CPB. Three out of five animals (60%) in the HBOC group compared to zero out of six (0%) in the no HBOC group required transient low-dose dopamine (3-5 ug/kg/min) to maintain MAP  $\geq$  55 mmHg in the period immediately after separation from bypass. At baseline, animals were mildly hypothermic but once bypass was initiated all animals returned to normothermia. There were no significant differences in core temperature between the two groups.

#### Global oxygen metabolism

The HBOC group had a significantly higher mean total hemoglobin level at baseline ( $9.7 \pm 0.7$  gm/dL vs.  $8.5 \pm 0.7$  gm/dL,  $p=0.02$ ) and this persisted throughout the protocol including post CPB ( $9.0 \pm 0.8$  gm/dL vs.  $7.6 \pm 0.7$  gm/dL,  $p=0.005$ ). Plasma hemoglobin was nearly undetectable at baseline - ( $0.1 \pm 0.1$  gm/dL in the HBOC group vs  $0.02 \pm 0.04$  gm/dL in the Blood group,  $p=NS$ ). In the HBOC group, mean plasma hemoglobin increased to  $2.2 \pm 0.3$  gm/dL ( $p=0.05$ ) compared to  $0.02 \pm 0.04$  gm/dL ( $p=NS$ ) in the Blood group, post CPB. Mean arterial oxygen content ( $CaO_2$ ) was also higher in the HBOC group at baseline and all subsequent time points (see Table 2). The mean  $CaO_2$  decreased from baseline in both groups once CPB was initiated due to hemo-dilution with the priming solution. At each interval, CPB flow changed as intended in the study design and did not vary significantly between groups at each time point. Despite the higher mean  $CaO_2$  in the HBOC group, calculated mean oxygen delivery ( $DO_2$ ) was not significantly different between the two groups but did decrease relative to baseline as mean CPB flow decreased. Calculated mean oxygen utilization ( $VO_2$ ) did not vary significantly between groups. At 50% CPB flow, the decrease

in mean  $\text{VO}_2$  became significant relative to the baseline value. Mixed venous saturation ( $\text{SVO}_2$ ) dropped significantly at 75% flow rate in both groups and remained decreased even after full flow had been resumed.

#### Global metabolic markers

Mean methemoglobin percentages were similar at baseline ( $0.7 \pm 0.4\%$  vs  $1.3 \pm 0.8\%$ ), remained at this level in the Blood group, and increased to a peak of  $2.3 \pm 0.8\%$  in the HBOC group vs.  $1.1 \pm 0.5\%$  in the Blood group ( $p < 0.001$ ). At all time points after initiating bypass, the methemoglobin percentage was significantly higher in the HBOC group. Mild metabolic acidosis developed in the Blood group as reflected by the mean arterial base excess and mean arterial pH (see Table 3). In the HBOC treated group, these parameters were not statistically different from baseline. In both groups, mean serum lactate levels were normal at baseline and increased as flow rate was decreased, but to a lesser degree in the HBOC group.

#### $^{15}\text{O}$ PET

Mean cerebral blood flow and mean cerebral metabolic rate of oxygen at 100% CPB flow was consistent with normal values for porcine brain. CBF was maintained at 75% CPB flow but decreased significantly from the full flow state in both groups when total CPB flow was decreased to 50% with no significant difference between the two groups (see Table 4). Once full flow was resumed, the values for mean CBF exceeded baseline. Mean  $\text{CMRO}_2$  was also maintained at 75% CPB flow in both groups. In the Blood group, the mean  $\text{CMRO}_2$  dropped significantly when total flow was reduced to 50%. In the HBOC group, the mean  $\text{CMRO}_2$  also decreased, but the decrease did not reach significance ( $p = 0.14$ ). Mean brain OEF increased as total flow, the change became significant in the HBOC group at 50% flow.

### Inflammatory Markers and Cardiac Enzymes

Serum levels of inflammatory markers, cardiac enzymes, magnesium and platelet levels are summarized in Table 5. All of these, with the exception of magnesium and TNF- $\alpha$  changed significantly from baseline to completion. Mean creatine kinase was elevated at baseline due to the sample being collected after opening of the chest and extremity cutdown. Electrocautery through skeletal muscle was performed during these procedures. Mean platelets levels decreased after the CPB run, but no significant difference between groups was identified.

### **Discussion-**

This is the first report of  $^{15}\text{O}$  PET scanning during cardiopulmonary bypass to evaluate cerebral blood flow and cerebral oxygen metabolism. In addition, the effects of HBOC-201 in the priming solution on hemodynamics, oxygen delivery and consumption, cerebral perfusion and oxygenation, and systemic inflammatory markers were evaluated. During CPB, the mean arterial pressure was slightly higher in the HBOC group consistent with the known mild vasoconstrictive properties of the HBOC. This effect may be beneficial during CPB when the use of vasoconstrictors to maintain an adequate perfusion pressure is often required. The need for low-dose inotropic support for a brief period after separation from CPB was unique to the HBOC group. The etiology is not clear, but the requirement was transient. The only prior study evaluating HBOC-201 during CPB (21) was performed in a canine model and the post-CPB left ventricular end-diastolic pressure and dP/dt measurements were unchanged from baseline suggesting left ventricular function was preserved. In our study, the animals were not instrumented to determine these parameters. Visual inspection of myocardial function was also impaired due to the positioning within the PET scanner. Pigs are prone to

development of pulmonary hypertension in the perioperative period following CPB. The potential contribution of pulmonary vasoconstriction and hypertension cannot be evaluated in our study. Future studies should further investigate the etiology underlying this transient need for inotropic/vasopressor support.

The mean hemoglobin concentration in the HBOC group was higher at baseline and throughout the study. The unexpectedly low mean baseline hemoglobin in the pigs compared to the HBOC-201 formulation contributed to this difference. HBOC-201 is a 13gm/dL solution (Table 5) and even though a similar volume of donor blood was used for priming the circuit in the non-HBOC group, the mean total hemoglobin was consistently 1.5 gm/dL higher in the HBOC group. The mean  $\text{CaO}_2$  is directly related to total hemoglobin and therefore, was also higher in the HBOC group. The mean  $\text{DO}_2$  was not significantly different between the groups but did decrease in a 1:1 relationship as the CPB flow was decreased. Mean  $\text{VO}_2$  seemed to be slightly higher in the HBOC group, but this difference was not significant. Mean  $\text{VO}_2$  was maintained at near baseline values at 75% flow by an increase in oxygen extraction in both groups. Mean  $\text{SvO}_2$  was also lower in the HBOC group but did not achieve statistical significance. Once CPB flow and  $\text{DO}_2$  was decreased to 50%, mean  $\text{VO}_2$  decreased significantly from baseline and mean mixed venous saturation was critically low in both groups. By maintaining the animals normothermic and decreasing the flow rate,  $\text{VO}_2$  became dependant on  $\text{DO}_2$ .

Mean methemoglobin levels were higher in the HBOC group, but with a peak level of  $2.3 \pm 0.8 \%$  there is no clinical significance. The enzyme, methemoglobin reductase, is located within erythrocytes and therefore unable to act on hemoglobin outside the cell. In the

Blood group, animals became mildly acidemic with higher peak lactate level and a slower decrease after normal flow was restored. Lactate is a product of cellular respiration during anaerobic metabolism. This pattern of lactate in the HBOC group suggests less anaerobic metabolism and more rapid clearance. This finding supports our hypothesis that during the low-flow state, improved oxygen unloading and tissue utilization leads to less anaerobic metabolism.

Mean cerebral blood flow was not significantly different between the two groups and autoregulation prevented a significant decrease in mean CBF until the CPB flow was decreased to 50%. When flow was restored to 100mL/kg/min, an increase in mean CBF was seen in both groups and has been described as reactive hyperemia after an ischemic insult. Similarly, at 75% flow, CMRO<sub>2</sub> was no different than full flow. When the flow was further reduced to 50%, only the Blood group decreased significantly. The mean CMRO<sub>2</sub> in the HBOC group decreased 16% while the Blood group decreased 29% relative to full flow. Once flow was restored, the mean oxygen metabolism returned to normal. These data further support the hypothesis that using HBOC-201 as an oxygen therapeutic during CPB can maintain cerebral oxygen metabolism during low-flow states by facilitating efficient oxygen utilization.

A number of the ischemia and inflammatory markers increased significantly on the average from baseline to post-CPB. This pattern was seen in total CK, CKMB, Troponin I, AST, and IL-6. In all of these, the mean increase was greater in the HBOC group. The difference between values at completion was only significantly higher in the HBOC group for CKMB, although troponin approached significance (p=0.08). Interestingly, mean TNF- $\alpha$ , did

not increase significantly during the experiment and there was no significant difference between groups. The etiology for these findings is unclear. Global mean oxygen delivery was similar for both groups and mean oxygen utilization was slightly higher in the HBOC group. Histopathology was not performed in this study. Previous studies found elevation in liver function tests after HBOC treatment without evidence of significant injury (7). The elevated troponin with HBOC has been previously reported in a canine model using crystalloid cardioplegia(21). In that study, the magnitude of elevation was much higher due to the global myocardial ischemia induced by aortic cross-clamp application, but followed a similar pattern. However, other models have found a myocardial protective effect of HBOC. In a canine model of experimental myocardial ischemia(11) using HBOC-201 as pretreatment, infarct size was smaller and cardiac enzyme elevation significantly less in the treatment group. In a rabbit model of coronary occlusion (10), both prophylactic and therapeutic administration of HBOC decreased the size of myocardial infarction without reducing the area of impaired perfusion. Elevated troponin following HBOC treatment continues to be a concern, but the clinical impact of the mild elevation in our study requires further evaluation.

Our study has several limitations. First, the difference in mean hemoglobin levels confounds the interpretation of the oxygen delivery and consumption data. Although the mean  $DO_2$  was not significantly different between the groups, the question arises: was the improved performance in the HBOC group the result of the HBOC properties or of the higher mean total hemoglobin? While this is a weakness of our research study, it is exactly how we expect this product would be used clinically. When adding HBOC-201 to the bypass circuit, the circulating free hemoglobin would capitalize on the unique properties in the intra-operative and immediate post-operative period when end-organ malperfusion is most likely.



The cell-free hemoglobin would be in addition to the RBC hemoglobin and not a substitute for transfusion. Second, using a healthy juvenile animal to evaluate end organ perfusion as a model for human patients with diffuse atherosclerosis is imperfect. Situations where the mechanism of decreased oxygen delivery is due to vascular occlusive disease rather than low CPB flow rates may behave differently than our model. Finally, the non-survival model does not allow for correlation of the immediate intraoperative findings to functional neurologic outcomes. Future investigations should utilize a survival model to evaluate the post-operative neurologic status and confirm that increased mean CMRO<sub>2</sub> during CPB leads to better clinical outcome.

### **Conclusion-**

In our porcine model, addition of HBOC-201 to the pump-priming solution during CPB improves cerebral oxygenation and decreases overall tissue ischemia compared to priming with donor whole blood. Further evaluation of immediate post-CPB myocardial function and increased cardiac enzymes after exposure to HBOC-201 should be performed prior to clinical application. The unique oxygen unloading properties have the potential to improve tissue oxygenation in numerous conditions of decreased oxygen delivery.

***Tables:***

Table 1. Properties of HBOC-201

13 g/dL polymerized hemoglobin  
    < 5% as unstabilized tetramers  
    ~ 50% between 65 and 130 kDa  
    < 10% are >500 kDa  
Colloid oncotic pressure 18 mm Hg  
 $t^{1/2}$ , 24–30 h  
pH, 7.8  
KCl, 4 mmol/L  
NaOH, 10 mmol/L  
p50, 38 mm Hg  
300 mOsm/kg  
NaCl, 113 mmol/L  
CaCl<sub>2</sub>, 1.4 mmol/L  
Sodium lactate, 27 mmol/L  
N-acetyl-L-cysteine, 200 mg/dL  
< 0.1 mcg/mL free glutaraldehyde  
< 0.05 EU/mL endotoxin

Table 2. Oxygen Delivery and Consumption

	HBOC-201 Prime(n=5)					Blood Prime(n=6)			
	100%	75%	50%	100%		100%	75%	50%	100%
Total Hg (gm/dL)	9.0 ± 0.6 <sup>Δ</sup>	8.7 ± 0.7 <sup>Δ</sup>	8.9 ± 1.3 <sup>Δ</sup>	8.4 ± 0.6*		7.4 ± 0.4*‡	7.4 ± 0.8*‡	7.4 ± 0.8*‡	7.2 ± 0.7*‡
CPB Flow (L/min)	2.3 ± 0.2	1.7 ± 0.1*	1.2 ± 0.1*	2.3 ± 0.2		2.4 ± 0.5	1.8 ± 0.3*	1.2 ± 0.2*	2.4 ± 0.4
CaO <sub>2</sub> (mL O <sub>2</sub> /dL)	12.8 ± 0.6 <sup>Δ</sup>	12.3 ± 0.9*	12.9 ± 1.6 <sup>Δ</sup>	11.8 ± 0.7*		10.9 ± 0.5*‡	10.9 ± 1.3*‡	10.9 ± 1.3*‡	10.4 ± 1.2*‡
SvO <sub>2</sub> (%)	61.5 ± 7.2	44.1 ± 7.3*	36.8 ± 6.4*	53.0 ± 6.3 <sup>Δ</sup>		65.4 ± 8.2	51.2 ± 9.7*	37.9 ± 9.5*	57.6 ± 10.1 <sup>Δ</sup>
DO <sub>2</sub> (mL O <sub>2</sub> /min)	293 ± 28	211 ± 29*	150 ± 29*	271 ± 30 <sup>Δ</sup>		257 ± 57	190 ± 34*	129 ± 26*	246 ± 58
VO <sub>2</sub> (mL O <sub>2</sub> /min)	122 ± 26	122 ± 21	99 ± 22*	134 ± 27		105 ± 38	95 ± 18	86 ± 28*	112 ± 29

\*p≤0.001 relative to initial full flow (100%), within group; <sup>Δ</sup>p<0.05 relative to initial full flow (100%), within group; ‡p<0.05 between groups  
CPB, cardiopulmonary bypass; CaO<sub>2</sub>, arterial oxygen content; SvO<sub>2</sub>, mixed venous oxygen saturation; DO<sub>2</sub>, oxygen delivery; VO<sub>2</sub>, oxygen consumption

Table 3. Acid/Base Measurements

	HBOC-201 Prime(n=4)						Blood Prime(n=6)					
	Baseline	100%	75%	50%	100%	Off CPB	Baseline	100%	75%	50%	100%	Off CPB
<b>BE</b> (mEq/l)	6.0 ± 4.1	6.4 ± 3.1	7.0 ± 4.1	3.9 ± 3.4	5.5 ± 2.7	4.1 ± 3.9	5.6 ± 3.4	7.6 ± 3.3 <sup>Δ</sup>	6.4 ± 3.5	2.0 ± 3.2 <sup>Δ</sup>	2.6 ± 4.1	4.2 ± 4.3
<b>pH</b>	7.44 ± 0.08	7.45 ± 0.09	7.48 ± 0.07	7.47 ± 0.07	7.44 ± 0.06	7.43 ± 0.06	7.45 ± 0.05	7.47 ± 0.06	7.46 ± 0.04	7.40 ± 0.05	7.39±0.06 <sup>Δ</sup>	7.41 ± 0.06
<b>pCO<sub>2</sub></b> (mmHg)	46 ± 5	45 ± 9	42 ± 3	38 ± 4 <sup>Δ</sup>	45 ± 4	44 ± 1	44 ± 5	44 ± 5	44 ± 4	43 ± 1	46 ± 3	47 ± 6
<b>Lactate</b> (mmol/l)	1.1± 0.4	2.2 ± 0.9*	2.5 ± 0.6*	4.5± 0.7*	3.4 ± 1.5*	3.3 ± 1.2*	1.5 ± .5	1.9 ± 0.7	3.2 ± 1.2*	6.5 ± 1.7*	6.3±2.4 <sup>‡</sup> *	4.7 ± 2.2*
<b>pO<sub>2</sub></b> (mmHg)	366 ± 134	282 ± 50	264 ± 58	339 ± 75	238 ± 55	319 ± 85	333 ± 131	337 ± 127	315 ± 120	350 ± 131	269 ± 149	257±178 <sup>Δ</sup>

\*p≤0.001 relative to baseline, within group; Δp≤0.05 relative to baseline, within group; ‡p=0.01 between groups

BE, arterial base excess; pH, arterial pH; pCO<sub>2</sub>, arterial partial pressure carbon dioxide; lactate, arterial lactate level; pO<sub>2</sub>, arterial partial pressure oxygen

Table 4. Cerebral Blood Flow and Oxygen Utilization

	HBOC-201 Prime(n=5)					Blood Prime(n=6)			
	100%	75%	50%	100%		100%	75%	50%	100%
CBF(ml/100g/min)	51.4 ± 8.3	44.8 ± 8.4	33.8 ± 10.7 <sup>Δ</sup>	63.4 ± 11.6		52.3 ± 11.8	53.0 ± 13.8	32.3 ± 11.6*	68.3 ± 11.5 <sup>Δ</sup>
CMRO <sub>2</sub> (ml/100g/min)	2.97 ± 0.72	2.85 ± 0.51	2.49 ± 0.36	3.1 ± 0.52		2.92 ± 0.96	3.17 ± 0.71	2.08 ± 0.65*	3.22 ± 0.76
OEF	0.46 ± 0.14	0.53 ± 0.11	0.61 ± 0.17 <sup>Δ</sup>	0.43 ± 0.11		0.56 ± 0.19	0.57 ± 0.15	0.63 ± 0.19	0.46 ± 0.13

\*p=0.004 relative to initial full flow (100%), within group; Δp=0.02 relative to initial full flow (100%), within group; ♦p=0.01 relative to initial full flow (100%), within group; ☆p=0.05 relative to initial full flow (100%), within group

CBF, cerebral blood flow; CMRO<sub>2</sub>, cerebral metabolic rate of oxygen; OEF, cerebral oxygen extraction fraction

Table 5. Inflammatory markers and cardiac enzymes

	HBOC-201 Prime			Blood Prime			
	Baseline	Completion	p (within group)	Baseline	Completion	p (within group)	p (between groups)
Total CK	928 ± 528	3414 ± 3104	0.001	589 ± 160	1653 ± 602	<0.001	NS
CKMB	10 ± 9	289 ± 348	0.02	5 ± 7	125 ± 290	0.08	0.05
Troponin I	0.1 ± 0.1	9.8 ± 3.8	<0.001	0.1 ± 0.1	4.6 ± 1.6	<0.001	0.08
AST	47 ± 9	252 ± 182	<0.001	41 ± 12	144 ± 70	<0.001	NS
BUN	8 ± 3	13 ± 3	<0.001	10 ± 4	16 ± 5	<0.001	NS
Magnesium	2.0 ± 0.5	2.2 ± 0.5	NS	1.8 ± 0.2	2.4 ± 0.2	0.003	NS
Platelets	313 ± 132	198 ± 52	0.05	333 ± 151	184 ± 33	0.004	NS
IL-6	101 ± 78	587 ± 765	0.02	46 ± 59	158 ± 87	0.09	0.07
TNF- $\alpha$	235 ± 187	253 ± 45	NS	138 ± 54	166 ± 78	NS	NS

CK, creatine kinase; CKMB, creatine kinase MB; AST, aspartate aminotransferase; BUN, blood urea nitrogen; IL-6, interleukin 6; TNF- $\alpha$ , tumor necrosis factor alpha.

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## References:

1. Bucci E. Thermodynamic approach to oxygen deliver in vivo by natural and artificial oxygen carriers. *Biophysical Chemistr.* 2009; 142:1-6.
2. Koehler RC, Fronticelli C, Bucci E. Insensitivity of cerebral oxygen transport to oxygen affinity of hemoglobin-based oxygen carriers. *Biochim Biophys Acta* 2008; 1784(10):1387-1394.
3. Tsoukias NM, Goldman D, Vadapalli A, Pittman RN, Popel AS. A computational model of oxygen delivery by hemoglobin-based oxygen carriers in three-dimensional microvascular networks. *Journal of Theoretical Biology* 2007; 248:657-674.
4. Dimino ML, Palmer AF. Hemoglobin-based O<sub>2</sub> carrier O<sub>2</sub> affinity and capillary inlet pO<sub>2</sub> are important factors that influence O<sub>2</sub> transport in a capillary. *Biotechnol Prog* 2007; 23(4):921-931.
5. Kavdia M, Pittman RN, Popel AS. Theoretical analysis of effects of blood substitute affinity and cooperativity on organ oxygen transport. *J Appl Physiol* 2002; 93:2122-2128.
6. McNeil JD, Smith DL, Jenkins DH, York GB, Josephs JJ. Hypotensive resuscitation using a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) leads to reversal of anaerobic metabolism. *J Trauma* 2001; 50:1063-1075.
7. York GB, Eggers JS, Smith DL, et al. Low-volume resuscitation with a polymerized bovine hemoglobin-based oxygen-carrying solution (HBOC-201) provides adequate tissue oxygenation for survival in a porcine model of controlled hemorrhage. *J Trauma* 2003; 55:372-885.
8. Sampson JB, Davis MR, Mueller DL, Kashyap VS, Jenkins DH, Kerby JD. A comparison of the hemoglobin-based oxygen carrier HBOC-201 to other low-volume resuscitation fluids in a model of controlled hemorrhagic shock. *J Trauma* 2003; 55:747-754.
9. Fitzpatrick CM, Biggs KL, Atkins BZ, et al. Prolonged low-volume resuscitation with HBOC-201 in a large-animal survival model of controlled hemorrhage. *J Trauma* 2005; 59:273-283.
10. Rempf C, Standl T, Schenke K, et al. Administration of bovine polymerized haemoglobin before and during coronary occlusion reduces infarct size in rabbits. *Br J Anaesth* 2009; 103:496-504.
11. Caswell JE, Strange MB, Ribber DM, Gibson MF, Cole P, Leffer DJ. A novel hemoglobin-based blood substitute protects against myocardial reperfusion injury. *Am J Physiol Heart Circ Physiol* 2005; 288:H1796-H1801.
12. King DR, Cohn SM, Proctor KG. Resuscitation with a hemoglobin-based oxygen carrier after traumatic brain injury. *J Trauma* 2005; 59:553-562.
13. Patel MB, Feinstein AJ, Saenz AD, Majetschak M, Proctor KG. Prehospital HBOC-201 after traumatic brain injury and hemorrhagic shock in swine. *J Trauma* 2006; 61:46-56.
14. Freitag M, Standl TG, Gottschalk A, et al. Enhanced central organ oxygenation after application of bovine cell-free hemoglobin HBOC-201. *Can J Anesth* 2005; 52(9):904-914.
15. Frackowiak RSJ, Lenzi GL, Jones T, Heather JD. Quantitative measure of regional cerebral blood flow and oxygen metabolism in man using <sup>15</sup>O and positron emission



- tomography: theory, procedure, and normal values. *J Comput Assist Tomogr* 1980; 4(6):727-736.
16. Poulsen PH, Smith DF, Ostergaard L, et al. In vivo estimation of cerebral blood flow, oxygen consumption and glucose metabolism in the pig by  $^{15}\text{O}$  water injection,  $^{15}\text{O}$  oxygen inhalation and dual injections of  $^{18}\text{F}$  fluorodeoxyglucose. *J Neuroscience Methods* 1997; 77:199-209.
  17. Mortberg E, Cumming P, Wiklund L, Rubertsson S. Cerebral metabolic rate of oxygen ( $\text{CMRO}_2$ ) in pig brain determine by PET after resuscitation from cardiac arrest. *Resuscitation* 2009; 80:701-706.
  18. National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press; 1996.
  19. Lee K, Fox PT, Lancaster JL, Jerabek PA. A positron-probe system for arterial input function quantification for positron emission tomography in humans. *Rev Sci Instrum*. 2008;79(6): 064301.
  20. Ohta S, Meyer E, Fujita H, et al. Cerebral  $^{15}\text{O}$  water clearance in humans determined by PET: I. Theory and normal values. *J Cereb Blood Flow Metab*. 1996;16(5):765-80.
  21. Neragi-Miandoab S, Guerrero JL, Vlahakes GJ. Autologous blood sequestration using a double venous reservoir bypass circuit and polymerized hemoglobin prime. *ASAIO Journal*. 2002;48: 407-411.
  22. Neragi-Miandoab S, Vlahakes GJ. Elevated troponin I level with hemoglobin based oxygen carrying solutions (HBOC) as a priming solution despite improved left ventricular function. *Interactive Cardiovascular and Thoracic Surgery*. 2006;5:135-8.

### Figures:

Figure 1. Heart rate & mean arterial pressure during cardiopulmonary bypass.

\* $p \leq 0.001$  between groups, @ $p < 0.001$  compared to baseline, # $p = 0.02$  compared to baseline

